Journal of the Formosan Medical Association (2013) 112, 382–389

Available online at www.sciencedirect.com

SciVerse ScienceDirect

journal homepage: www.jfma-online.com

ORIGINAL ARTICLE

Bcl-6 expression and lactate dehydrogenase level predict prognosis of primary gastric diffuse large B-cell lymphoma

Kun-Ming Chung [a], Sheng-Tsung Chang [b,l], Wen-Tsung Huang [c], Chin-Li Lu [d], Hung-Chang Wu [e], Wei-Shou Hwang [f,g], Kwang-Yu Chang [h,i,j], Shih-Sung Chuang [b,k,*]

[a] Department of Internal Medicine, Chi-Mei Medical Center, Tainan, Taiwan
[b] Department of Pathology, Chi-Mei Medical Center, Tainan, Taiwan
[c] Division of Hemato-Oncology, Department of Internal Medicine, Chi-Mei Medical Center, Liouying, Tainan, Taiwan
[d] Department of Medical Research, Chi-Mei Medical Center, Tainan, Taiwan
[e] Division of Hemato-Oncology, Department of Internal Medicine, Chi-Mei Medical Center, Tainan, Taiwan
[f] Division of Hematology and Oncology, Department of Internal Medicine, Chung-Shan Medical University Hospital, Taichung, Taiwan
[g] School of Medicine, Chung-Shan Medical University, Taichung, Taiwan
[h] National Institute of Cancer Research, National Health Research Institutes, Tainan, Taiwan
[i] Division of Haematology and Oncology, Department of Internal Medicine, National Cheng-Kung University Hospital, Tainan, Taiwan
[j] College of Medicine, National Cheng-Kung University, Tainan, Taiwan
[k] Department of Pathology, Taipei Medical University, Taipei, Taiwan
[l] Department of Nursing, National Tainan Institute of Nursing, Tainan, Taiwan

Received 19 January 2012; received in revised form 17 July 2012; accepted 23 July 2012

KEYWORDS
bcl-6;
diffuse large B-cell lymphoma;
germinal center B-cell phenotype;
primary gastric lymphoma;

Background/Purpose: The gastrointestinal tract is the most common site of primary extranodal non-Hodgkin lymphoma, and the prognostic factors of primary gastric diffuse large B-cell lymphoma (PG-DLBCL) differ in various studies.
Methods: We retrospectively searched for PG-DLBCL in a single institution, performed immunohistochemical analysis, classified tumor phenotype (Hans and Muris algorithms), reviewed medical records, and analyzed the clinical and immunophenotypic variables using Cox proportional hazard regression model.

* Corresponding author. Department of Pathology, Chi-Mei Medical Center, 901, Chung-Hwa Road, Yung-Kang District, Tainan 710, Taiwan.
E-mail address: cmh5301@mail.chimei.org.tw (S.-S. Chuang).

http://dx.doi.org/10.1016/j.jfma.2012.07.031

stomach;
Taiwan

Results: A total of 46 cases were identified including 25 males and 21 females with a median age of 63.5; 18 (39%) were at stage I and 28 (61%) at stage II. Seven (15%) patients underwent surgery as initial treatment including total ($n = 3$, 7%) and subtotal ($n = 4$, 9%) gastrectomy. Thirty-three patients (72%) received frontline chemotherapy treatment including ten with additional rituximab (MabThera) injection, and two (6%) of these patients developed perforation after chemotherapy. Four patients passed away shortly after diagnosis and the remaining three were lost to follow-up. The overall 2- and 5- year survival rates were 55% and 50%, respectively. The expression of various differentiation markers was CD10 (25%), bcl-2 (50%), bcl-6 (84%), and MUM1 (64%). Half of the cases studied (22/44) were classified as germinal center B-cell (GCB) phenotype and the remaining half as non-GCB according to Hans algorithm; 66% and 34% cases belonged to groups 1 and 2, respectively, according to Muris algorithm. Univariate analysis showed the expression of bcl-6 by the tumor cells as a favorable factor, while elevated serum lactate dehydrogenase (LDH) level, bcl-2 expression, and Muris group 2 were associated with poorer outcome. Multivariate analysis revealed that the two prognostic factors were bcl-6 expression and elevated LDH level, with hazard ratios of 0.09 ($p = 0.002$) and 3.72 ($p = 0.024$), respectively.
Conclusion: In this retrospective study with heterogeneous treatment modality, we identified bcl-6 expression and elevated LDH level as two prognostic factors for PG-DLBCL.

Introduction

The gastrointestinal tract is the most common organ for primary extra-nodal non-Hodgkin lymphoma (NHL).[1] Most primary gastrointestinal lymphomas occur in the stomach with mucosa-associated lymphoid tissue (MALT) lymphoma and diffuse large B-cell lymphoma (DLBCL) as the most frequent histological types.[2–5] DLBCL is the most common type of lymphoma worldwide and is clinically and biologically heterogeneous.[6] Clinically, patients differ in their mode of presentation and respond variably to therapy. A combination of clinical parameters such as Eastern Cooperative Oncology Group performance status (PS), Ann Arbor stage, lactate dehydrogenase (LDH) level, and International Prognostic Index (IPI) can be used to predict response to therapy and survival.[7] Pathologically, DLBCL differ in morphology, immunophenotype, and genetic features. Numerous markers detectable by immunohistochemistry and linked to different aspects of tumor biology have been studied in DLBCL including more recently, stage-specific markers of B-cell differentiation.[8]

There have been several studies on primary gastric (PG) MALT lymphomas including those with high-grade transformation from Taiwan, yet there are scanty reports on PG-DLBCL.[9–11] In a previous study of 30 cases with primary intestinal DLBCL from Taiwan, we identified perforation as the only poor prognostic factor.[12] In this retrospective study, we aimed at elucidating the clinicopathological features of PG-DLBL in Taiwan in the hope of identifying the prognostic factors.

Material and methods

We retrospectively searched the lymphoma database for gastric DLBCL from January 1994 to December 2008 at the Chi-Mei Medical Center, Tainan, Taiwan. Strict inclusion criteria for PG lymphoma were applied according to the American Joint Committee on Cancer Staging Manual, which is a modification of the Ann Arbor System.[13] We excluded cases of PG MALT lymphomas with high-grade transformation and systemic lymphomas with secondary gastric involvement. This study was approved by our Institutional Review Board.

Specimens were preserved in 10% formalin, processed by routine methods, and embedded in paraffin. All of the original hematoxylin and eosin—stained sections (HE-stained sections) and/or newly cut and HE-stained sections were reviewed. Immunohistochemical staining on 4-μm sections was performed for each case with either the labeled streptavidin—biotin peroxidase method (LSAB kit, Dako Corp., Carpinteria, CA, USA) or a polymer-based detection system (Bond Polymer Refine Detection, Vision BioSystems Ltd., Newcastle Upon Tyne, UK), and an antigen-retrieval technique was applied as needed for each antibody. The antibodies used were CD3, CD20, CD21, bcl-2, bcl-6, IRF4/MUM1 (MUM1p), Ki-67 (MIB-1) (DakoCytomation, Glostrup, Denmark), and CD10 (Novocastra, Newcastle upon Tyne, UK). Appropriate positive controls were used for all immunohistochemical staining. For the interpretation of CD10, bcl-2, bcl-6, and IRF4/MUM1, positive signals $\geq$30% of neoplastic cells were considered to be positive. We applied the algorithms of Hans et al and Muris et al to categorize the cases into germinal center B-cell (GCB) versus non-GCB phenotype and groups 1 versus 2, respectively, for prognostic stratification.[14,15]

Staging procedure included computed tomography, magnetic resonance imaging and/or positron emission tomography scans of the chest and abdomen, and bone marrow aspiration and biopsy. Patients were staged according to the criteria proposed by the International Workshop for gastrointestinal NHL.[16] Medical records of the patients were reviewed. Progression-free survival rate was defined as the time from diagnosis to progression, relapse or death from any cause. The overall survival (OS) rate was measured from the date of diagnosis to the date of last follow-up or death. Cox proportional hazard regression analyses were performed to estimate the hazard ratio (HR)

of predictors. Kaplan—Meier survival curves were drawn and log-rank test was used to compare differences between survival curves. All statistical analyses were performed using SPSS version 12.0.7 for Windows (SPSS Inc., Chicago, IL, USA). A p value < 0.05 was considered to be statistically significant.

Results

We identified 53 patients with gastric DLBCL from initial search. One patient with stage III and four patients with stage IV disease were excluded, as we could not definitely differentiate between primary and secondary gastric lymphomas. Two patients with coexisting MALT lymphoma were also excluded from our study. Therefore, only the remaining 46 cases were included in this study. Table 1 summarizes the pertinent clinicopathological findings of these patients, including 25 males and 21 females (M:F = 1.2:1). The median age was 63.5 years with a range of 14—87 years. Eighteen (39%) patients were at stage I and 28 (61%) were at stage II. Seven (15%) patients underwent surgery as initial treatment including total (n = 3, 7%) and subtotal (n = 4, 9%) gastrectomy. Thirty-three patients (72%) received combination chemotherapy with COP [cyclophosphamide, vincristine, (Oncovin) and prednisolone; 10 patients (22%)] or CHOP [cyclophosphamide, doxorubicin, vincristine (Oncovin) and prednisolone; 23 patients (50%)] as frontline therapy. One patient in the COP-treated group and nine patients in CHOP-treated groups were also treated with rituximab. Of these 33 patients, 3 subsequently underwent subtotal gastrectomy due to gastric outlet obstruction (Case 32) or perforation (Cases 19 and 26; 6%). Of these two patients, one died of this complication 10 days later (Case 19), while the other was free of disease for 88 months when examined at the last follow-up (Case 26). Two patients received radiotherapy, including Patient No. 30 as salvage therapy for relapsed tumor (4600 cGy to neck nodes and 5040 cGy to para-aortic nodes and spleen) and No. 36 as adjuvant therapy (4200 cGy) following R-CHOP. Four patients passed away shortly after biopsy (0.3—2.0 months) without chemotherapy either due to old age, poor PS, or severe co-morbidity; three patients were lost to follow-up.

We stratified the nonsurgically treated patients into three groups: supportive care without chemotherapy, chemotherapy with either COP or CHOP-like regimens. The OS curves of these three groups of patients are depicted in Fig. 1. The only statistically significant comparison was between COP and CHOP (p = 0.025; log-rank test), while there was no statistical significance between no chemotherapy and COP (p = 0.784) or no chemotherapy and CHOP (p = 0.073) groups. The median OS time of patients receiving CHOP-based chemotherapy was 61 months. The progression-free and OS curves are depicted in Fig. 2. The median progression-free and OS time were 17 and 46 months, respectively. The 2- and 5- year OS rates of the entire cohort were 55.3% (95% CI: 40.8—69.8%) and 50.0% (95% CI: 35.1—64.9%), respectively.

Histopathologically, all tumors showed diffuse infiltration of medium to large atypical lymphocytes without lymphoepithelial lesion or any low-grade component (Fig. 3). Immunophenotypically, all tumors expressed CD20 but not CD3. Staining with anti-CD21 identified no follicular dendritic meshworks. No tumor tissue was left for immunohistochemical study of differentiation antigens in the two cases (Cases 6 and 12). Of the remaining 44 cases, the expression of various differentiation markers in a decreasing order was bcl-6 (84%), MUM1 (64%), bcl-2 (50%), and CD10 (25%). According to the algorithm of Hans et al, half of the cases (22/44) were classified as GCB and the remaining half as non-GCB.[14] According to the criteria of Muris et al, 66% and 34% cases were groups 1 and 2, respectively.[15]

Table 2 lists the prognostic significance of various clinical and laboratory parameters, differentiation markers, and phenotypic grouping using univariate Cox proportional hazard regression model. Bcl-6 expression was associated with a favorable outcome (HR: 0.23; p = 0.004), while elevated LDH level (HR: 3.20; p = 0.031), bcl-2 expression (HR: 2.57; p = 0.043), and Muris group 2 (HR: 2.43; p = 0.048) were associated with poorer prognosis. The other B-cell differentiation antigens (CD10 and MUM1), Ki-67 labeling index (≧80% vs. <80%), and GCB versus non-GCB by Hans algorithm were not related to prognosis. All the other clinical parameters including gender, age at diagnosis (≧60 vs. <60), diagnostic method (biopsy vs. subtotal or total gastrectomy), PS (0—1 vs. 2—4), IPI score (0—1 vs. 2—5), and stage (I vs. II) were of no prognostic significance. All variables in the univariate analysis with $p < 0.2$ were included for multivariate analysis using Cox proportional hazard regression model, while bcl-6 expression (HR: 0.09; 95% CI: 0.02—0.42; p = 0.002) and elevated LDH level (HR: 3.72; 95% CI: 1.19—11.68; p = 0.024) remained prognostically significant.

Discussion

Previous series have identified various clinical prognostic parameters such as early-stage detection, younger age, and radical surgery in patients with PG lymphoma.[17—19] In these studies, however, patients with diseases with low-grade component (MALT lymphoma) and/or with high-stage disease had been included. The survival probability for patients with gastric MALT lymphomas is significantly better than patients with secondary high-grade transformation or *de novo* PG-DLBCL; whereas the survival of the latter two high-grade tumor groups is usually not significantly different.[20] In our study focusing only on patients with pure PG-DLBCL at low-stage diseases, we found that the two prognostic variables were bcl-6 expression by the tumor cells and elevated LDH level but not other clinical parameters. In a study of Hong Kong—Chinese population with PG-DLBCL and gastric MALT lymphoma with high-grade component, high bcl-6 expression predicted better prognosis, independent of *BCL6* translocation status, translocation partner, or *BCL6*-deregulation mutations.[21] In a Japanese study of 29 cases of pure PG-DLBCL without a low-grade component, the expression rate of CD10 (41%), bcl-2 (28%), and bcl-6 (76%) was comparable to ours (25%, 50%, and 84%, respectively); and bcl-6 expression was one of two favorable prognostic factors in addition to stage

Table 1 Clinical and laboratory parameters, immunohistochemistry, and phenotype in primary gastric DLBCL.

No./gender/age	Stage	Dx method	C/T	ECOG-PS	IPI	Immunohistochemistry					Hans	Muris	PFS (m)	Outcome (m)
						CD10	bcl-2	bcl-6	MUM1	Ki-67				
1/F/49	II	Biopsy	COP	1	NA	−	+	+	+	70	Non-GCB	2	9	DOD (10)
2/M/63	II	Subtotal	Nil	1	NA	−	−	+	−	60	GCB	1	120	NED (120)
3/M/65	I	Biopsy	CHOP	1	NA	+	−	+	+	80	GCB	1	0	DOD (3)
4/M/71	I	Biopsy	Nil	3	NA	−	+	+	−	50	GCB	1	0	LTF (8.5)
5/F/60	I	Subtotal	Nil	1	1	−	+	+	+	90	Non-GCB	2	43	DOD (43)
6/F/66	II	Biopsy	COP	1	NA	ND	ND	ND	ND	ND	NA	NA	44	DOD (46)
7/F/59	I	Total	Nil	1	NA	−	−	+	−	40	GCB	1	27	DOD (28)
8/F/76	I	Biopsy	Nil	3	NA	−	−	+	+	70	GCB	1	0	LTF (10)
9/F/80	II	Subtotal	Nil	1	NA	+	+	+	+	90	GCB	1	7	DOD (7)
10/F/51	II	Biopsy	CEOP	1	1	+	−	+	−	95	GCB	1	0	DOD (6)
11/F/58	II	Biopsy	COP	1	1	−	+	−	−	80	Non-GCB	1	0	DOD (2)
12/F/65	II	Biopsy	CEOP	1	1	ND	ND	ND	ND	ND	NA	NA	8	DOD (9)
13/F/82	II	Biopsy	COP	2	NA	−	+	−	+	40	Non-GCB	2	0	DOD (2)
14/M/81	I	Biopsy	Nil	4	NA	−	+	−	−	90	GCB	1	0	DOD (1.5)
15/M/35	II	Biopsy	CEOP	0	2	+	−	−	+	90	Non-GCB	1	0	DOD (4)
16/M/52	II	Biopsy	CEOP	0	1	−	−	+	−	40	GCB	1	20	DOD (22)
17/F/52	I	Biopsy	CEOP	1	1	−	−	+	+	40	Non-GCB	1	122	NED (122)
18/M/48	II	Biopsy	CEOP	1	0	−	−	+	−	ND	GCB	1	111	NED (111)
19/F/65[a]	II	Biopsy	COP	1	1	−	+	+	+	ND	Non-GCB	2	0	DOD (6)
20/M/14	II	Biopsy	CEOP	1	0	+	−	+	−	70	GCB	1	106	NED (106)
21/F/73	II	Biopsy	Nil	4	NA	−	+	+	+	ND	Non-GCB	2	0	DOD (0.3)
22/F/50	II	Subtotal	R-CHOP	1	0	−	+	+	−	ND	GCB	1	92	NED (92)
23/F/61	I	Biopsy	Nil	0	NA	−	−	+	+	ND	Non-GCB	1	0	LTF (1)
24/F/75	II	Biopsy	COP	1	1	−	+	−	+	ND	Non-GCB	2	0	DOD (5)
25/M/41	II	Biopsy	CEOP	1	0	+	−	+	−	100	GCB	1	91	NED (91)
26/M/66[a]	I	Biopsy	CEOP	1	1	−	−	+	−	100	GCB	1	88	NED (88)
27/M/62	I	Biopsy	CEOP	1	2	−	+	+	+	80	Non-GCB	2	70	DOD (75)
28/M/76	II	Biopsy	COP	1	1	ND	−	+	+	95	Non-GCB	1	80	NED (80)
29/M/62	II	Biopsy	R-CHOP	1	2	+	+	+	+	60	Non-GCB	2	85	NED (85)
30/F/55[b]	II	Biopsy	CEOP	1	0	−	−	+	+	80	Non-GCB	1	35	AWD (91)
31/M/34	I	Biopsy	R-CHOP	1	0	+	+	+	+	95	GCB	1	73	NED (73)
32/M/69[a]	II	Biopsy	R-CHOP	2	2	−	−	+	−	100	GCB	1	42	DOUD (42)
33/M/57	I	Biopsy	CHOP	1	0	−	−	+	−	90	GCB	1	64	NED (64)
34/M/58	I	Biopsy	CHOP	1	0	−	+	+	+	90	Non-GCB	2	61	NED (61)
35/M/65	II	Biopsy	COP	3	3	−	−	+	−	90	GCB	1	0	DOD (1)
36/M/48[b]	I	Biopsy	R-CHOP	1	0	−	−	+	+	90	Non-GCB	1	60	NED (60)
37/M/68	I	Biopsy	Nil	4	3	−	+	−	+	90	Non-GCB	2	0	DOD (2)
38/F/82	I	Total	Nil	1	NA	−	+	−	+	90	Non-GCB	2	54	NED (54)
39/F/76	II	Biopsy	Nil	4	3	−	+	+	+	90	Non-GCB	2	0	DOD (2)
40/M/51	II	Biopsy	R-CHOP	2	4	+	−	+	+	90	GCB	1	0	DOD (4.2)
41/F/77	I	Biopsy	R-COP	1	2	−	−	+	−	90	GCB	1	68	NED (68)
42/M/73	II	Total	Nil	2	3	+	+	+	+	90	GCB	1	0	DOD (1.5)
43/F/76	II	Biopsy	COP	2	3	−	+	+	+	90	Non-GCB	2	0	DOD (3.5)
44/M/79	I	Biopsy	R-CHOP	2	3	−	+	+	+	90	Non-GCB	2	7	DOD (7)
45/M/87	II	Biopsy	R-CHOP	1	2	+	−	+	+	90	GCB	1	30	DOUD (30)
46/M/61	I	Biopsy	R-CHOP	1	1	−	+	+	+	70	Non-GCB	2	38	NED (38)

AWD = alive with disease; C/T = chemotherapy; COP = cyclophosphamide, vincristine (Oncovin), and prednisolone; CEOP = cyclophosphamide, epirubicin, vincristine (Oncovin), and prednisolone; CHOP = cyclophosphamide, doxorubicin, vincristine (Oncovin), and prednisolone; Dx = diagnosis; DOD = died of disease; DOUD = died of unrelated disease; ECOG-PS = Eastern Cooperative Oncology Group performance status; GCB = germinal center B-cell phenotype; IPI = International Prognostic Index; Ki-67, labeling index (%) as determined by Ki-67 immunohistochemistry; LTF = lost to follow-up; NA = not available; ND = not done; NED = no evidence of disease; PFS = progression-free survival; R-COP = rituximab plus COP; R-CHOP = rituximab plus CHOP; subtotal = subtotal gastrectomy; Total = total gastrectomy.

[a] These three patients received chemotherapy as initial treatment, developed gastric outlet obstruction (Case 32) or perforation (Cases 19 and 26) and subsequently underwent subtotal gastrectomy.

[b] These two patients received radiotherapy: No. 30 as savage therapy for relapsed tumors; No. 36 as adjuvant therapy following R-CHOP.

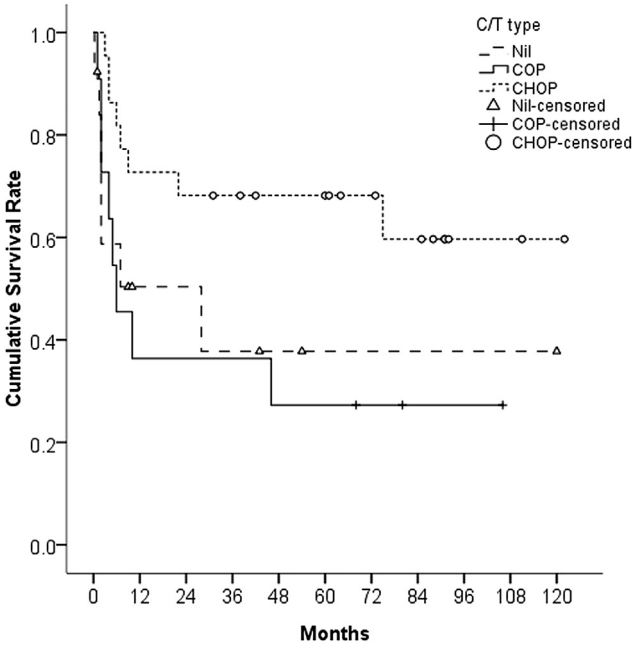

Figure 1 Overall survival curves of patients with primary gastric diffuse large B-cell lymphoma stratified by no chemotherapy, and chemotherapy with COP and CHOP, respectively.

classification of the disease.[22] Bcl-6, a marker of GCB origin, is encoded by *BCL6* proto-oncogene located at the 3q24 locus with pivotal roles in germinal center formation and regulation of lymphocyte function, differentiation, and survival. *BCL6* is a frequently activated oncogene in the pathogenesis of human B-cell lymphoma, most of which are derived from GCB.[23,24] The expression of bcl-6 protein has been associated with a favorable prognosis in DLBCL.[8,25] The prognostication of DLBCL in the rituximab era might be different from the earlier prognostic models.[26] Winter et al showed that in bcl-6-negative DLBCL patients, failure-free survival (FFS) and OS rates were prolonged in those treated only with R-CHOP compared to CHOP treatment alone. In contrast, no differences in FFS and OS rates were detected between treatment arms for bcl-6-positive cases.[27] In our study, the number of patients treated with R-CHOP was too small for a meaningful statistical analysis. Prospective studies on treatment using R-CHOP are warranted to examine whether bcl-6 expression retains its prognostic value in PG-DLBCL.

DLBCL is a heterogeneous disease.[6] Using a complementary DNA microarray method, DLBCLs could be divided into prognostically important subgroups: GCB-like and non-GCB-like.[28,29] Patients with GCB-like DLBCL had a significantly better OS rate than those with non-GCB type. Hans et al reported the first immunohistochemical algorithm as a surrogate method for genetic profiling to predict prognostic impact in DLBCL and the effectiveness of their algorithm has been proved in various studies.[14,30,31] Subsequently, Muris et al used immunohistochemical profiling based on bcl-2, CD10, and IRF4/MUM1 expression, yet its prognostic utility is conflicting, particularly when rituximab is included in the chemotherapeutic regimen.[15,27,32] Very recently, Meyer et al compared

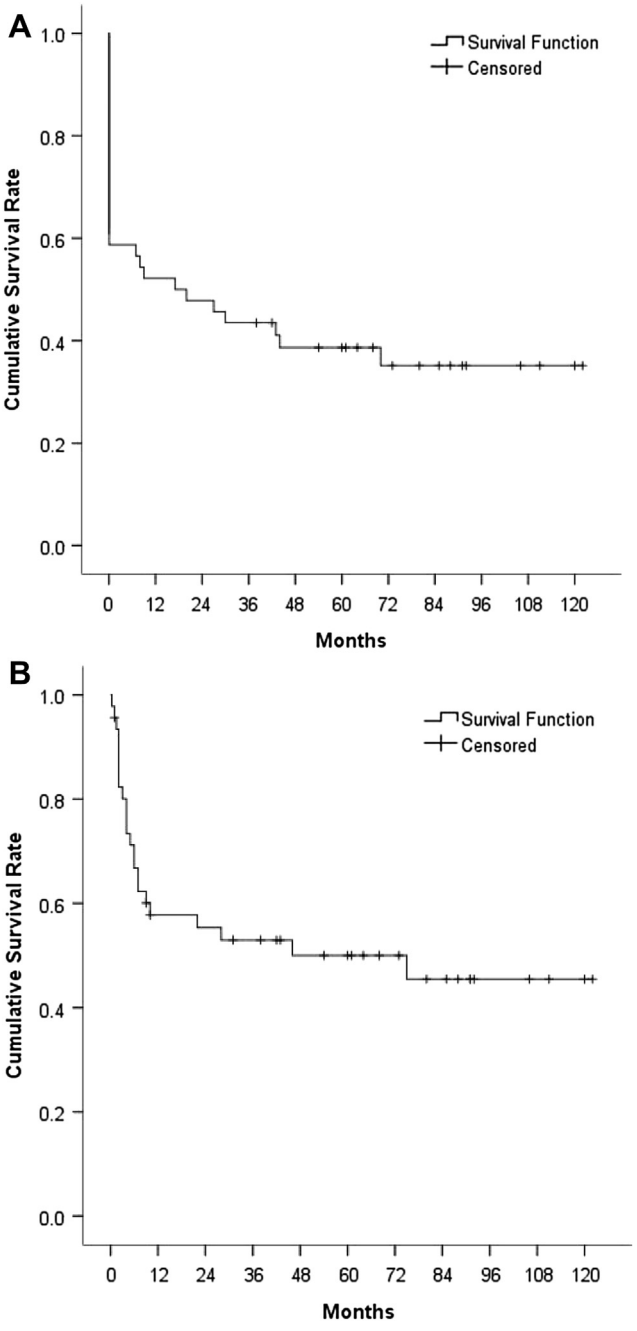

Figure 2 (A) Progression-free survival and (B) overall survival of patients with primary gastric diffuse large B-cell lymphoma.

various algorithms on a large cohort of DLBCL patients treated with R-CHOP or R-CHOP-like therapy.[33] They found that Hans and Choi algorithms had high concordance with the microarray results and Tally algorithm showed the best concordance with the microarray data while maintaining the prognostic significance and ease of use with only four antibodies (CD10, MUM1, GECT1, and FoxP1) without regard to the order of examination.[33] In a recent study on *de novo* DLBCL by Gutiérrez-García et al comparing gene expression profiling (GEP) and immunohistochemical algorithms, the GEP groups showed significantly different better 5-year progression-free survival and OS rates in the GCB group than the activated B-cell group; in contrast, none of the

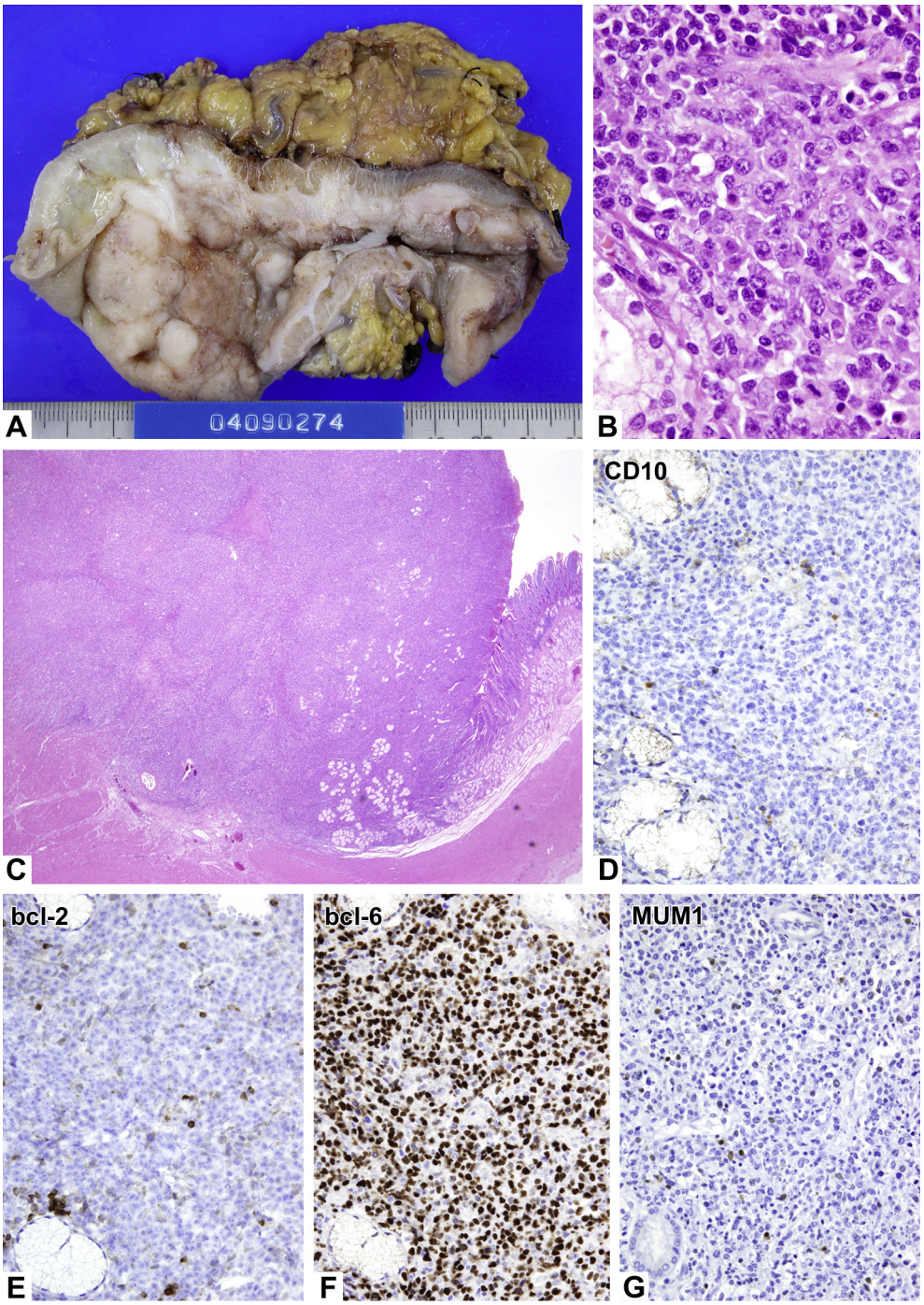

Figure 3 A representative case of primary gastric lymphoma. (A) and (C) Gross and microscopic scanning power showing a polypoid tumorous lesion with transmural infiltration of lymphoma cells. (B) High-power view shows diffuse infiltration of large lymphoma cells with centroblastic morphology and gastric epithelial cells at the left-lower corner. The tumor cells express bcl-6 (F) but not CD10 (D), bcl-2 (E) or MUM1 (G), a GCB phenotype by Hans algorithm and type 1 by Muris algorithm.

immunohistochemical algorithms (Colomo, Hans, Muris, Choi, and Tally) was able to retain the prognostic impact of the groups (GCB vs. non-GCB).[34] While in patients with relapsed/refractory DLBCL, Thieblemont et al showed that the GCB phenotype based on the Hans algorithm was significantly associated with a better progression-free survival in the rituximab, dexamethasone, high-dose cytarabine, and cisplatin (R-DHAP) arm.[35] Whether immunohistochemical algorithms could be used as surrogate markers for GEP is controversial and is a subject of debate; however, as pointed out by Gutiérrez-García et al, stratification of DLBCL cases based on immunostaining algorithms should be used with caution in guiding therapy, even in clinical trials.[34]

The algorithm of Hans et al is the most widely applied, probably in part because the markers used (CD10, bcl-6,

Table 2 Univariate Cox proportional hazard regression model for prognostic markers in primary gastric diffuse large B-cell lymphoma.

Variables	HR	95% CI		*p*
		Lower	Upper	
Gender (M vs. F)	0.54	0.24	1.25	0.150
Age (>60 vs. ≦60)	2.02	0.83	4.93	0.122
Dx method (surgery vs. Bx)	0.67	0.20	2.27	0.525
LDH (elevated vs. normal)	3.20	1.11	9.17	0.031
Stage (II vs. I)	1.35	0.87	2.07	0.178
ECOG-PS (2—4 vs. 0—1)	1.25	0.78	2.00	0.359
IPI (0—1 vs. 2—5)	0.46	0.16	1.34	0.156
C/T (yes vs. no)	0.60	0.25	1.45	0.270
CD10 (positive vs. negative)	1.14	0.44	2.95	0.784
bcl-2 (positive vs. negative)	2.57	1.03	6.41	0.043
bcl-6 (positive vs. negative)	0.23	0.09	0.62	0.004
MUM1 (positive vs. negative)	1.69	0.65	4.37	0.282
Ki-67 index (≧80 vs. <80)	0.81	0.53	1.25	0.340
Hans et al (GCB vs. non-GCB)	1.78	0.58	5.42	0.312
Muris et al (group 2 vs. 1)	2.43	1.01	5.87	0.048

Bx = biopsy; C/T = chemotherapy; CI = confidence interval; Dx = diagnosis; ECOG-PS = Eastern Cooperative Oncology Group performance status; GCB = germinal center B-cell; HR = hazard ratio; IPI = International Prognostic Index; LDH = lactate dehydrogenase.

and MUM1/IRF4) are readily available in the majority of pathology laboratories, which are useful in the differential diagnosis of Burkitt lymphoma in conjunction with bcl-2 and Ki-67.[14,36] In this study, there was an equal distribution of GCB (50%) and non-GCB phenotypes in PG-DLBCL. In our previous study of 30 patients with surgically resected primary intestinal DLBCLs, the proportion of GCB phenotype was 30%, showing no prognostic impact of differentiation antigens or GCB and non-GCB phenotypes based on the Hans algorithm.[12] In a recent British study of PG and intestinal DLBCLs using the same algorithm, 27% (4/15 cases) of gastric tumors were of GCB type as compared with 64% (9/14) of intestinal tumors.[37] In that study, the differentiation antigens or GCB versus non-GCB phenotype did not carry any prognostic significance in either the PG or intestinal DLBCL groups. Another study from the US also revealed differences in GCB versus non-GCB phenotype in PG [58% (14/24 cases) GCB] and intestinal [88% (19/22) GCB] DLBCL groups; again, there was no significant difference in either OS or disease-free survival between the GCB and non-GCB groups.[38] These reports indicate a difference in the distribution of GCB versus non-GCB phenotype in gastric and intestinal DLBCL among various geographic regions, possibly reflecting the impact of ethnic and environmental factors in lymphomagenesis. Furthermore, the differentiation antigens or Hans algorithm did not have a prognostic impact, suggesting that other factors may play important roles for prognosis.

Perforation is a frequent presentation of primary intestinal lymphomas in Taiwan, particularly in those with T-cell phenotype.[12,39] As T-cell lymphomas are generally more aggressive than their B-cell counterparts, the higher rate of perforation might be an indicator of biological aggressiveness.[12,39] While none of the patients presented with perforation in our current study, the occurrence of acute gastric perforation or gastrointestinal hemorrhage in patients with PG lymphoma is a recognized rare complication of chemotherapy, with a rate of <5% in most studies.[40,41] In our study, 2 of 33 patients (6%) developed gastric perforation after frontline chemotherapy and 1 passed away 10 days after undergoing subtotal gastrectomy. Although perforation is a rare complication, Maisey et al suggested that routine admission for the initiation of chemotherapy for PG lymphoma was not necessary and all patients should receive comprehensive education about the risks and clinical signs of gastric perforation and bleeding.[41]

In summary, we characterized the clinicopathological features of PG-DLBCL in Taiwan, where the outcome was poor, partly due to old age, high PS scores, and comorbidity of some patients. Although this was a retrospective study with a limited case number and heterogeneous treatment modalities, we identified bcl-6 expression as a favorable prognostic marker and elevated LDH level as a poor prognosticator. In the other retrospective study involving a total of 423 consecutive DLBCL patients from May 1989 to December 2010 at our institution, we found bcl-6 protein expression to be a favorable prognostic factor ($p = 0.017$; multivariate Cox proportional hazard regression model) in addition to age (Chang et al, manuscript in preparation). Prospective studies are warranted to examine whether bcl-6 expression and LDH level retain their prognostic significance in PG-DLBCL in the rituximab era.

Acknowledgments

Drs Chung KM and Chang ST contributed equally to this work and both should be considered as first author. This work was supported by a research grant from the National Science Council (Grant No. NSC101-2314-B-384-006), Taipei, Taiwan.

References

1. Isaacson PG, Du MQ. Gastrointestinal lymphoma: where morphology meets molecular biology. *J Pathol* 2005;**205**:255—74.
2. Freeman C, Berg JW, Cutler SJ. Occurrence and prognosis of extranodal lymphomas. *Cancer* 1972;**29**:252—60.
3. Weingrad DN, Decosse JJ, Sherlock P, Straus D, Lieberman PH, Filippa DA. Primary gastrointestinal lymphoma: a 30-year review. *Cancer* 1982;**49**:1258—65.
4. Dragosics B, Bauer P, Radaszkiewicz T. Primary gastrointestinal non-Hodgkin's lymphomas. A retrospective clinicopathologic study of 150 cases. *Cancer* 1985;**55**:1060—73.
5. Zinzani PL, Magagnoli M, Pagliani G, Bendandi M, Gherlinzoni F, Merla E, et al. Primary intestinal lymphoma: clinical and therapeutic features of 32 patients. *Haematologica* 1997;**82**:305—8.
6. Stein H, Warnke RA, Chan WC, Jaffe ES. Diffuse large B-cell lymphoma, not otherwise specified. In: Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, et al., editors. *WHO classification of tumours of haematopoietic and lymphoid tissues*. Lyon: IARC; 2008. p. 233—7.
7. Offit K, Lo Coco F, Louie DC, Parsa NZ, Leung D, Portlock C, et al. Rearrangement of the bcl-6 gene as a prognostic marker in diffuse large-cell lymphoma. *N Engl J Med* 1994;**331**:74—80.

8. de Leval L, Harris NL. Variability in immunophenotype in diffuse large B-cell lymphoma and its clinical relevance. *Histopathology* 2003;**43**:509—28.
9. Chuang SS, Lee C, Hamoudi RA, Liu H, Lee PS, Ye H, et al. High frequency of t(11;18) in gastric mucosa-associated lymphoid tissue lymphomas in Taiwan, including one patient with high-grade transformation. *Br J Haematol* 2003;**120**:97—100.
10. Kuo SH, Chen LT, Yeh KH, Wu MS, Hsu HC, Yeh PY, et al. Nuclear expression of BCL10 or nuclear factor kappa B predicts *Helicobacter pylori*-independent status of early-stage, high-grade gastric mucosa-associated lymphoid tissue lymphomas. *J Clin Oncol* 2004;**22**:3491—7.
11. Liu TY, Dei PH, Kuo SH, Lin CW. Early low-grade gastric MALToma rarely transforms into diffuse large cell lymphoma or progresses beyond the stomach and regional lymph nodes. *J Formos Med Assoc* 2010;**109**:463—71.
12. Chuang SS, Ye H, Yang SF, Huang WT, Chen HK, Hsieh PP, et al. Perforation predicts poor prognosis in patients with primary intestinal diffuse large B-cell lymphoma. *Histopathology* 2008; **53**:432—40.
13. Edge SB, Byrd DR, Compton CC, Firtz AG, Greene VL, Trotti A. Lymphoid neoplasms, *AJCC cancer staging manual*. New York: Springer; 2010. 599—628.
14. Hans CP, Weisenburger DD, Greiner TC, Gascoyne RD, Delabie J, Ott G, et al. Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. *Blood* 2004;**103**:275—82.
15. Muris JJ, Meijer CJ, Vos W, van Krieken JH, Jiwa NM, Ossenkoppele GJ, et al. Immunohistochemical profiling based on Bcl-2, CD10 and MUM1 expression improves risk stratification in patients with primary nodal diffuse large B cell lymphoma. *J Pathol* 2006;**208**:714—23.
16. Rohatiner A, d'Amore F, Coiffier B, Crowther D, Gospodarowicz M, Isaacson P, et al. Report on a workshop convened to discuss the pathological and staging classifications of gastrointestinal tract lymphoma. *Ann Oncol* 1994;**5**: 397—400.
17. Colucci G, Naglieri E, Maiello E, Marzullo F, Caruso ML, Leo S, et al. Role of prognostic factors in the therapeutic strategy of primary gastric non Hodgkin's lymphomas. *Clin Ter* 1998;**149**: 25—30.
18. Hsi ED, Eisbruch A, Greenson JK, Singleton TP, Ross CW, Schnitzer B. Classification of primary gastric lymphomas according to histologic features. *Am J Surg Pathol* 1998;**22**: 17—27.
19. Mäkelä J, Karttunen T, Kiviniemi H, Laitinen S. Clinicopathological features of primary gastric lymphoma. *J Surg Oncol* 1999;**70**:78—82.
20. Cogliatti SB, Schmid U, Schumacher U, Eckert F, Hansmann ML, Hedderich J, et al. Primary B-cell gastric lymphoma: a clinicopathological study of 145 patients. *Gastroenterology* 1991; **101**:1159—70.
21. Chen YW, Hu XT, Liang AC, Au WY, So CC, Wong ML, et al. High BCL6 expression predicts better prognosis, independent of BCL6 translocation status, translocation partner, or BCL6-deregulating mutations, in gastric lymphoma. *Blood* 2006; **108**:2373—83.
22. Takeshita M, Iwashita A, Kurihara K, Ikejiri K, Higashi H, Udoh T, et al. Histologic and immunohistologic findings and prognosis of 40 cases of gastric large B-cell lymphoma. *Am J Surg Pathol* 2000;**24**:1641—9.
23. Barrans SL, Evans PA, O'Connor SJ, Kendall SJ, Owen RG, Haynes AP, et al. The t(14;18) is associated with germinal center-derived diffuse large B-cell lymphoma and is a strong predictor of outcome. *Clin Cancer Res* 2003;**9**:2133—9.
24. Basso K, Dalla-Favera R. BCL6: master regulator of the germinal center reaction and key oncogene in B cell lymphomagenesis. *Adv Immunol* 2010;**105**:193—210.
25. Lin CH, Kuo KT, Chuang SS, Kuo SH, Chang JH, Chang KC, et al. Comparison of the expression and prognostic significance of differentiation markers between diffuse large B-cell lymphoma of central nervous system origin and peripheral nodal origin. *Clin Cancer Res* 2006;**12**:1152—6.
26. Ninan MJ, Wadhwa PD, Gupta P. Prognostication of diffuse large B-cell lymphoma in the rituximab era. *Leuk Lymphoma* 2011;**52**:360—73.
27. Winter JN, Weller EA, Horning SJ, Krajewska M, Variakojis D, Habermann TM, et al. Prognostic significance of Bcl-6 protein expression in DLBCL treated with CHOP or R-CHOP: a prospective correlative study. *Blood* 2006;**107**:4207—13.
28. Alizadeh AA, Eisen MB, Davis RE, Ma C, Lossos IS, Rosenwald A, et al. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. *Nature* 2000;**403**:503—11.
29. Rosenwald A, Wright G, Chan WC, Connors JM, Campo E, Fisher RI, et al. The use of molecular profiling to predict survival after chemotherapy for diffuse large-B-cell lymphoma. *N Engl J Med* 2002;**346**:1937—47.
30. Berglund M, Thunberg U, Amini RM, Book M, Roos G, Erlanson M, et al. Evaluation of immunophenotype in diffuse large B-cell lymphoma and its impact on prognosis. *Mod Pathol* 2005;**18**:1113—20.
31. Sjö LD, Poulsen CB, Hansen M, Møller MB, Ralfkiaer E. Profiling of diffuse large B-cell lymphoma by immunohistochemistry: identification of prognostic subgroups. *Eur J Haematol* 2007; **79**:501—7.
32. Yamauchi A, Fujita S, Ikeda J, Nakamichi I, Fukuhara S, Hino M, et al. Diffuse large B-cell lymphoma in the young in Japan: a study by the Osaka Lymphoma Study Group. *Am J Hematol* 2007;**82**:893—7.
33. Meyer PN, Fu K, Greiner TC, Smith LM, Delabie J, Gascoyne RD, et al. Immunohistochemical methods for predicting cell of origin and survival in patients with diffuse large B-cell lymphoma treated with rituximab. *J Clin Oncol* 2011;**29**:200—7.
34. Gutiérrez-García G, Cardesa-Salzmann T, Climent F, González-Barca E, Mercadal S, Mate JL, et al. Gene-expression profiling and not immunophenotypic algorithms predicts prognosis in patients with diffuse large B-cell lymphoma treated with immunochemotherapy. *Blood* 2011;**117**:4836—43.
35. Thieblemont C, Briere J, Mounier N, Voelker HU, Cuccuini W, Hirchaud E, et al. The germinal center/activated B-cell subclassification has a prognostic impact for response to salvage therapy in relapsed/refractory diffuse large B-cell lymphoma: a bio-CORAL study. *J Clin Oncol* 2011;**29**:4079—87.
36. Chuang SS, Ye H, Du MQ, Lu CL, Dogan A, Hsieh PP, et al. Histopathology and immunohistochemistry in distinguishing Burkitt lymphoma from diffuse large B-cell lymphoma with very high proliferation index and with or without a starry-sky pattern: a comparative study with EBER and FISH. *Am J Clin Pathol* 2007;**128**:558—64.
37. Connor J, Ashton-Key M. Gastric and intestinal diffuse large B-cell lymphomas are clinically and immunophenotypically different. An immunohistochemical and clinical study. *Histopathology* 2007;**51**:697—703.
38. Mitchell KA, Finn WG, Owens SR. Differences in germinal centre and non-germinal center phenotype in gastric and intestinal diffuse large B-cell lymphomas. *Leuk Lymphoma* 2008;**49**:1717—23.
39. Chuang SS, Chang ST, Chuang WY, Huang WT, Hsieh PP, Tsou MH, et al. NK-cell lineage predicts poor survival in primary intestinal NK-cell and T-cell lymphomas. *Am J Surg Pathol* 2009;**33**:1230—40.
40. Al-Akwaa AM, Siddiqui N, Al-Mofleh IA. Primary gastric lymphoma. *World J Gastroenterol* 2004;**10**:5—11.
41. Maisey N, Norman A, Prior Y, Cunningham D. Chemotherapy for primary gastric lymphoma: does in-patient observation prevent complications? *Clin Oncol (R Coll Radiol)* 2004;**16**:48—52.